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Antibody persistence and booster response in adolescents and young adults 4 and 7.5 years after immunization with 4CMenB vaccine

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ABSTRACT

Background: Data on duration of protection against invasive meningococcal disease post-vaccination with the recombinant, 4-component, meningococcal serogroup B vaccine (4CMenB) are limited. We evaluated bactericidal activity persistence in adolescents/young adults up to 7.5 years post-primary vaccination with 4CMenB, and response to a booster dose compared with vaccine-naïve controls.
Methods: This open-label, multicenter study (NCT02446743) enrolled 15–24 year-old-previously vaccinated participants from Canada, Australia (group Primed_4y) 4 years post-priming with 4CMenB (2 doses; 0,1-month schedule), and Chile (Primed_7.5y) 7.5 years after priming with 4CMenB (2 doses; 0,1/0,2/0,6-month schedule) and vaccine-naïve participants of similar age (Naïve_4y and Naïve_7.5y groups). Primed participants received a booster dose; vaccine-naïve participants received 2 catch-up doses of 4CMenB, 1 month apart. We evaluated antibody persistence and immune responses using hSBA in terms of geometric mean titers and percentages of participants with hSBA titers $\geq$4, the kinetics of bactericidal activity post-booster (previously vaccinated) or post-2 doses (vaccine-naïve), and safety.
Results: Antibody levels declined at 4 (Primed_4y) and 7.5 (Primed_7.5y) years post-primary vaccination, but remained higher than in vaccine-naïve participants at baseline ($\leq$44% vs $\leq$ 13% [fHbp]; $\leq$84% vs $\leq$ 24% [NadA]; $\leq$29% vs $\leq$ 14% [PorA]) for all vaccine antigens except NHBA ($\leq$81% vs $\leq$ 79%). One month post-booster and post-second dose, 93–100% of primed and 79–100% of vaccine-naïve participants had hSBA titers $\geq$4 for all antigens. Kinetics of the antibody response were similar across groups with an early robust response observed 7 days post-booster/second dose. No vaccine-related serious adverse event was reported.

Abbreviations: 4CMenB, 4-component serogroup B recombinant meningococcal vaccine; AE, adverse event; CI, confidence interval; FAS, full analysis set; fHbp, factor H-binding protein; GMR, geometric mean ratio; GMT, geometric mean titer; hSBA, human complement serum bactericidal antibody assay; IMD, invasive meningococcal disease; MenB, *Neisseria meningitidis* serogroup B; NadA, Neisseria adhesin A; NHBA, Neisserial heparin-binding antigen; PorA, porin A; PPS, per-protocol set; SAE, serious adverse event.

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Conclusion: For all antigens except NHBA, a higher proportion of primed participants had hSBA titers ≥4, at 4 and 7.5 years post-vaccination, compared with vaccine-naïve participants. A more robust immune response after booster compared to a first dose in vaccine-naïve individuals, showed effective priming in an adolescent/young adult population. No safety or new reactogenicity issues were identified.

An Audio Summary linked to this article that can be found on Figshare: https://figshare.com/articles/Antibody_persistence_and_booster_response_in_adolescents_and_young_adults_4_and_7_5_years_after_immunization_with_4CMenB_vaccine/9437276; https://doi.org/10.6084/m9.figshare.9437276.v1.

1. Introduction

Widespread use of serogroup-specific vaccines against *Neisseria meningitidis* including serogroups A, C, W and Y has significantly decreased the incidence of invasive meningococcal disease (IMD) caused by these serogroups [1,2]. *N. meningitidis* serogroup B (MenB) remains a major cause of outbreaks and endemic disease worldwide [1,2]. IMD incidence is highest in infants <1 year of age, but a second, smaller peak is reported in adolescents in whom meningococcal carriage predominantly occurs [3]. In developed countries, overall IMD mortality rates can reach 10% and many survivors experience physical and neurological sequelae, despite treatment [2].

IMD incidence varies globally, ranging between <0.5 cases/100,000 in North America to 10 cases/100,000 in the African meningitis belt [2]. In recent years, reported IMD incidence was 0.77 cases/100,000 population in Australia (2015) [4], 0.60 cases/100,000 in Canada (2014) [5] and 0.80 cases/100,000 in Chile (2015) [6]. Before 2010, MenB was responsible for >60% of the total IMD cases reported in Australia [4,7], Canada [5,8] and Chile [6]. However in the last decade, MenB incidence has declined in these countries, while MenW incidence has increased in Australia [7] and Chile [9].

The development of a 4-component serogroup B meningococcal vaccine (4CMenB, *Bexsero*, GSK) and the MenB:fHBP vaccine *(Trumenba*, Pfizer) represents a significant recent advance in IMD prevention [10]. 4CMenB contains 3 recombinant protein antigens (*Neisseria* adhesin A [NadA], Neisserial heparin binding antigen [NHBA] and factor H-binding protein [fHbp]) and outer membrane vesicles (OMV) expressing porin A protein (PorA), components that can provide broad protection against the majority of IMD caused by MenB strains [10].

However, evaluating the efficacy of MenB vaccines is challenging, due to the low IMD incidence and therefore, to the difficulty in obtaining real-life data. Moreover, the variability of surface protein antigens in the sequence and level of expression with MenB strains is well known [11]. Clinical trial designs use laboratory assays to estimate antibody activity against antigen-specific meningococcal reference strains. The threshold used for MenB evaluation is a human complement serum bactericidal antibody assay (hSBA) titer of at least 4, accepted as a correlate of protection for serogroup C [12], and extended to the other *N. meningitidis* serogroups.

The target population for immunization against IMD varies worldwide, with available vaccines against 5 meningococcal serogroups, and the possibility to vaccinate different age groups [13,14]. In case of outbreak or exposure, the level of circulating antibody providing direct protection is an important consideration because of the rapid onset of IMD. Some countries have introduced meningococcal vaccines in their routine infant and child vaccination programs to directly target the group most impacted by IMD [15]; however, due to the waning of meningococcal antibodies over time, these vaccines might not be able to offer protection through adolescence, when the second incidence peak usually emerges. Complete information on circulating antibody levels, booster response, and the effect of priming in all age groups is therefore of the outmost importance for vaccine scheduling.

4CMenB has been shown to be immunogenic and well-tolerated in adolescents [16–20] and is currently licensed in the United States as a 2-dose schedule in 10–25-year-olds, and for use from 2 months of age in Europe, Australia, Canada, and Latin America (Argentina, Brazil, Chile, Colombia and Uruguay) [10,21]. In September 2015, 4CMenB was introduced in the UK National Infant Immunization Program, where, 10 months after implementation, 4CMenB had halved the number of MenB IMD cases in vaccine-eligible infants [22].

The immunogenicity of 4CMenB in adolescents aged 11–17 years has been previously assessed in 3 studies [16–18] generating data for antibody persistence up to 2 years post-vaccination. In Chile [16], 2 doses administered 1, 2, or 6 months apart elicited protective immune responses in 98–100% of adolescents up to 1 month post-second dose. Additionally, seroprotective activity for all vaccine-related antigens (hSBA titers above pre-specified values) was maintained over at least 18–23 months post-second dose in 77–94% of vaccinated adolescents [18]. Consistent results were obtained in the third study, which evaluated lot-to-lot consistency of 2 batches, immunogenicity and safety of 4CMenB in healthy Australian and Canadian adolescents [17]. Similar results have been obtained in the infant population, with protective antibody levels for the different vaccine antigens ranging between 36% and 93% among infants at 24–36 months following vaccination according to different schedules in the first year of life [23].

Due to the rapid onset, high case-mortality and severe sequelae of IMD, the quick mounting and long-term persistence of protective levels of circulating antibodies is key for the successful prevention of disease. Although 4CMenB has been shown to be immunogenic and safe with protective circulating antibody levels up to 2 years, data on the duration of antibody levels after that period are limited. Here, we assessed the persistence of bactericidal activity in adolescents and young adults, at approximately 4 and 7.5 years after a 2-dose primary series of 4CMenB compared with serum bactericidal activity in vaccine-naïve participants of similar age. In addition we evaluated the response to a booster dose in participants from the parent studies [16,17], compared with primary vaccination in vaccine-naïve healthy controls.

Fig. 1 summarizes the research, clinical relevance and impact on the patient population.

2. Methods

2.1. Study design and participants

This was a phase IIIb, open-label, controlled, multicenter study (NCT02446743), conducted between November 2015 and September 2016 in 12 centers in Australia, Canada and Chile. In the parent study, adolescents and young adults aged 11–17 years received a primary vaccination of 2 doses of 4CMenB approximately 4 (Canada and Australia, NCT01423084) and 7.5 years (Chile, NCT00661713) before participating in this study [16,17]. In the present study, primed individuals aged 15–24 years were invited to receive a third, booster dose of 4CMenB and were enrolled in the Primed_4y group (previously vaccinated participants from

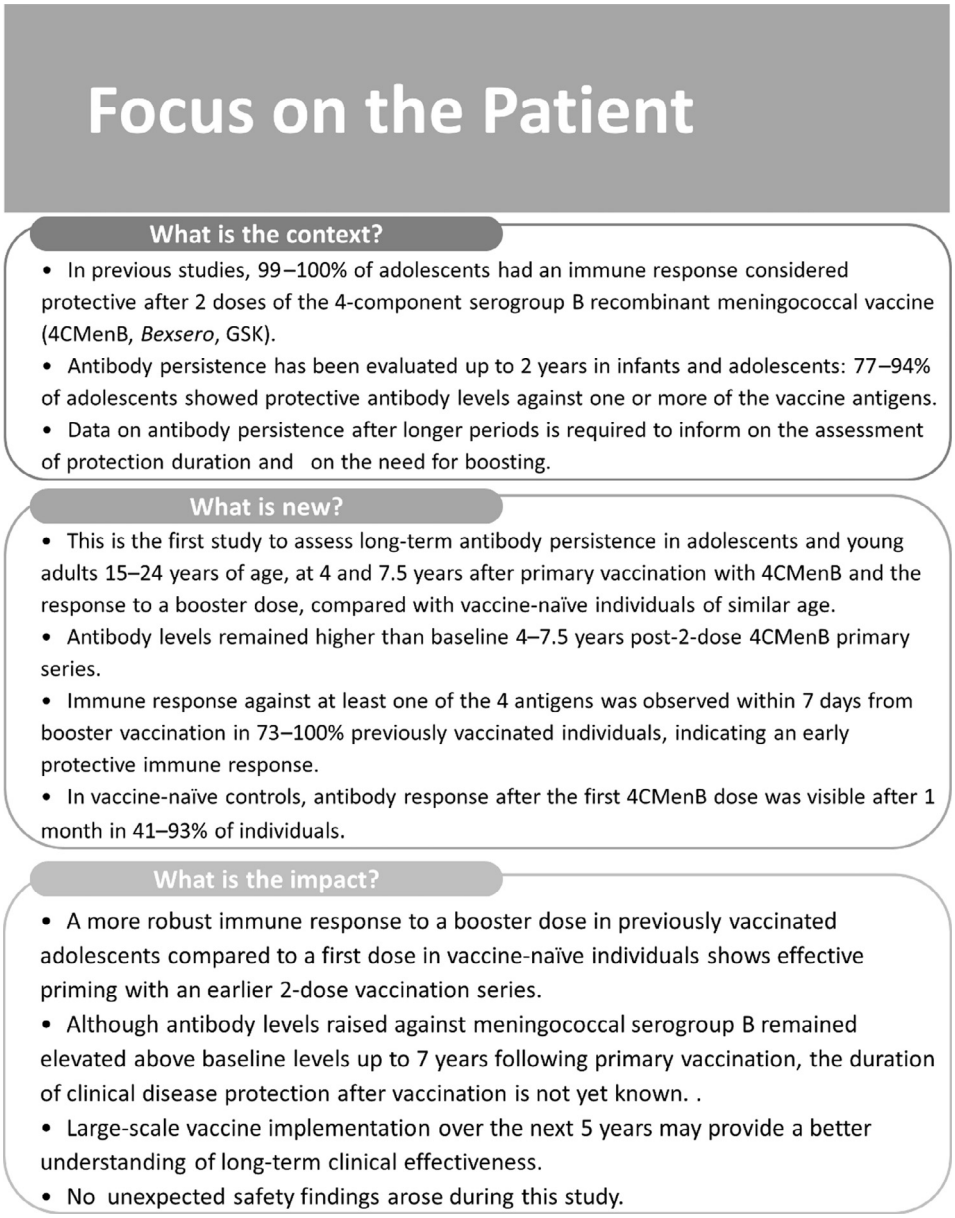

Fig. 1. Focus on the Patient Section.

Canada and Australia) and Primed_7.5y group (previously vaccinated participants from Chile).

Two groups of vaccine-naïve participants, Naïve_4y (naïve participants from Canada and Australia) and Naïve_7.5y (naïve participants from Chile) groups of similar age to Primed_4y and Primed_7.5y received 2 doses of 4CMenB, administered 1 month apart (Fig. 2).

A full list of inclusion and exclusion criteria is provided in Supplementary Text 1.

Previously vaccinated participants were not randomized to treatment. Vaccine-naïve participants were randomized in a 1:1 ratio for differential blood drawing in order to determine antibody kinetics. The first group had blood drawn at 3 and 30 days post-second dose; and the second group had blood drawn at 7 and 30 days post-second dose. Pre-first dose and post-dose 1 blood samples were also taken from vaccine-naïve participants. These data served as a control for kinetics evaluation post-booster dose in previously vaccinated participants from whom 4 blood samples were taken at 4 and 7.5 years post-primary vaccination (pre-booster, 3, 7 and 30 days post-booster dose). The blood samples collected from previously vaccinated adolescents at 1 month post-vaccination in the primary studies were also used as control in this study.

Written informed consent was obtained from participants (or parents/legal guardians of participants under 18 years of age) prior to enrollment in the study, and assent was obtained from all participants under 18 years of age. The protocol and the proposed informed consent form were reviewed and approved by the local Institutional Review Boards and/or an Ethics Committees before study start.

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. This study is registered at www.clinicaltrials.gov (NCT02446743). A summary of the protocol is available at http://www.gsk-clinicalstudyregister.com (study ID 205218).

2.2. Study objectives

The primary immunogenicity objective was to assess the persistence of serum bactericidal activity at 4 and 7.5 years following

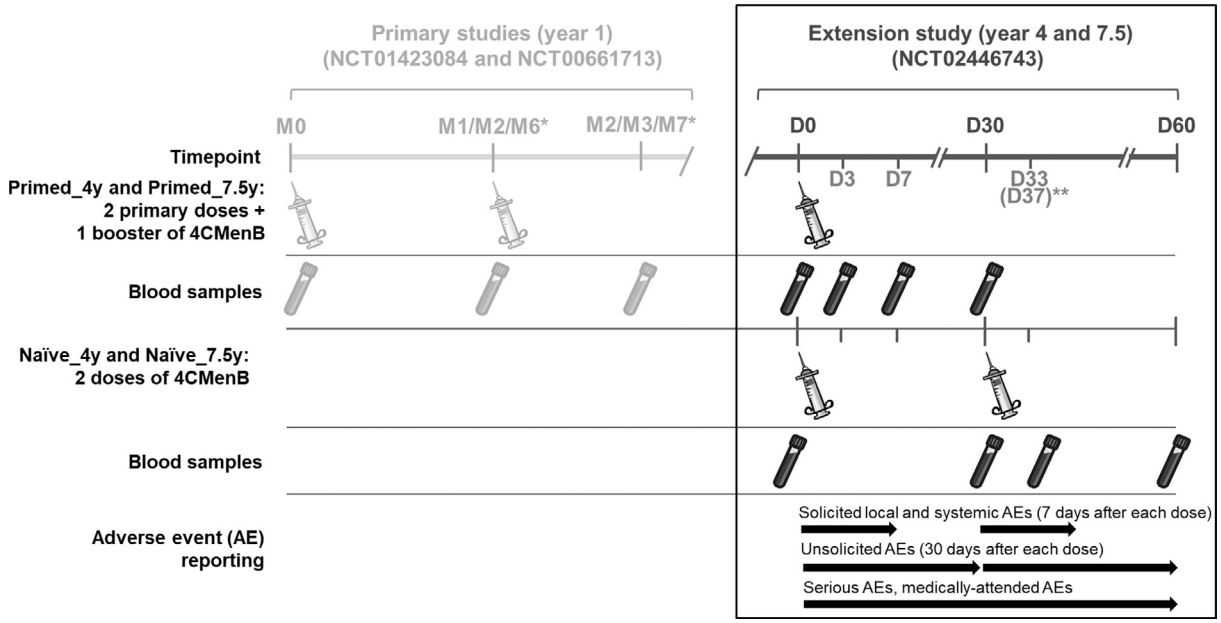

Fig. 2. Study design. M, month in the primary study; D, day in the extension study. Primed_4y, previously vaccinated participants from Canada and Australia; Naïve_4y, vaccine-naïve participants from Canada and Australia; Primed_7.5y, previously vaccinated participants from Chile; Naïve_7.5y, vaccine-naïve participants from Chile. The rectangle indicates the current study. Note: * Participants in group Naïve_4y received 2 primary doses, 1 month apart; participants in group Primed_7.5y received 2 primary doses, according to 1 of 3 schedules: M0,1; M0,2 and M0,6. Blood samples were collected before the first dose and 1 month post-second dose in each case. ** Participants in Groups Naïve_4y and Naïve_7.5y were randomized in a 1:1 ratio; half of participants had blood drawn at D0, D30 (1 month post-dose 1), D33 (3 days post-dose 2) and D60 (1 month post-dose 2), and the other half at D0, D30, D37 (7 days post-dose 2) and D60.

primary series compared to vaccine-naïve participants. The primary safety objective was to evaluate the safety and reactogenicity of 4CMenB in all participants.

Secondary immunogenicity objectives were: (i) to assess the immune response 1 month post-booster in previously vaccinated participants, compared to the immune response at 1 month post-first dose in vaccine-naïve participants; (ii) to assess and compare the response of the booster with the second dose administered to vaccine-naïve participants, in terms of kinetics at 3, 7 and 30 days post-vaccination; (iii) to assess the immune response of 4CMenB administered to adolescent and young adult vaccine-naïve participants, older than in the initial study (15–24 years of age), at 1 month post-dose 2.

2.3. Vaccines

The 4CMenB vaccine (lot number JB148101) contains 50 μg each of recombinant proteins NHBA, NadA, and fHbp, adsorbed on aluminum hydroxide, 25 μg OMV expressing PorA P1.4 from the serogroup B strain NZ98/254, aluminum hydroxide, sodium chloride, histidine and sucrose. 4CMenB suspension was prepared as a 0.5-mL dose and administered intramuscularly into the deltoid region of the non-dominant arm.

2.4. Immunogenicity assessment

A minimum of 10 mL of blood was drawn from each participant in the previously vaccinated groups at 3, 7 and 30 days post-booster dose and, for each participant in the vaccine-naïve groups at day 30, 33 (or 37) and 60 post-first dose (Fig. 2, Supplementary Fig. 1).

The immune response was measured against antigen-specific *N. meningitidis* MenB indicator strains H44/76 (fHbp), 5/99 (NadA), NZ98/254 (PorA) and M10713 (NHBA); the assay was previously described in detail [24]. Human plasma was used as the source of exogenous complement. Data were summarized by calculating the percentages of participants with hSBA titer ≥4, hSBA geometric mean titers (GMTs), and geometric mean ratio (GMRs) of GMTs post-booster vs pre-booster (previously vaccinated participants) or post-dose 1 vs pre-first dose (vaccine-naïve participants). The percentage of participants with hSBA titer ≥5, ≥8 and ≥16 was also calculated; results for the latter 2 cut-offs are available on ClinicalTrials.gov. Testing was conducted in a blinded manner with respect to the study group, at the GSK Clinical Laboratory Sciences (Marburg, Germany) or at the Charles River Laboratories Edinburgh Ltd., UK.

The percentages of participants with 4-fold increase from pre-vaccination to post-vaccination for the vaccine-naïve individuals were also summarized.

2.5. Safety assessment

Solicited local (injection site induration, swelling, erythema and pain) and systemic (fever [≥38.0 °C], high fever [≥39.5 °C], nausea, fatigue, myalgia, arthralgia, and headache) adverse events (AEs) were recorded using diary cards within 7 days post-vaccination and unsolicited AEs were reported within 30 days post-vaccination. The severity of the reported AEs and the relationship to the study vaccination were determined by the investigator.

Medically-attended AEs (MAEs) and serious AEs (SAEs) were collected throughout the study by interviewing the participants and/or parents/guardians, and by reviewing medical records.

2.6. Statistical analysis

The sample size for the previously vaccinated participants was determined by the number of participants from the parent studies eligible to participate in this extension study (344 for the Primed_4y group and 529 for the Primed_7.5y group); a sample size of maximum 400 participants was planned. A target sample size of 250 participants in the vaccine-naïve control group was determined to be large enough to reflect important variations in the

population, but small enough to accommodate the operational constraints common to the extension studies and to allow for reliable (>90% probability) observations of common (≥1%) AEs in the vaccine-naïve participants. For a sample size of 250 participants in the vaccine-naïve group, the probability of detecting at least one participant with an AE occurring with a frequency of 1% was 92%. No statistical hypotheses were associated with the immunogenicity objectives.

Persistence analyses (primary immunogenicity objective) were performed on the full analysis set (FAS), including participants for whom evaluable immunogenicity results were available for at least 1 antigen, pre-booster/first vaccination in the extension study. Post-booster/first dose analyses were conducted in the FAS booster, which included participants who received a booster/first dose of 4CMenB and for whom evaluable immunogenicity results were available for at least 1 antigen, at 1 month post-booster/first dose. Immune response kinetics analyses were performed on the per-protocol set (PPS), comprising participants who correctly received the vaccine, had no protocol deviations and had evaluable immunogenicity results at each timepoint.

The percentages of participants with hSBA titer ≥4 and associated 2-sided 95% confidence intervals (CIs) were computed for all participants, as well as differences in percentages and 95% CIs between the previously vaccinated group and vaccine-naïve participants. Unadjusted and adjusted GMTs and associated 95% CIs were computed for each group and for each vaccine antigen by exponentiating the least square means and the lower and upper limits of the 95% CIs of the log transformed titers or concentrations (base 10). Additionally, the GMT ratio of the previously vaccinated group to the vaccine-naïve group was computed. The 95% CIs for the ratio of GMTs were constructed by exponentiating the difference of the least square means of the log-transformed titers and the lower and upper limits of the 95% CI. In addition, data were summarized by calculating the percentage of participants with a 4-fold rise post-vaccination and associated 2-sided 95% CIs, for each vaccine antigen.

The safety analysis was performed on the 'as treated' analysis set. The incidence of AEs per study group was tabulated with exact 95% CIs after each vaccination.

3. Results

3.1. Demographics

A total of 531 individuals were enrolled, of whom 276 were previously vaccinated (Primed_4y: 145, Primed_7.5y: 131) and 255 were vaccine-naïve (Naïve_4y: 105, Naïve_7.5y: 150). All, except 1 previously vaccinated participant, were included in the FAS for persistence; 271 primed participants and 250 naïve participants completed the study (Supplementary Fig. 1).

Demographic and other characteristics at enrolment were balanced across previously vaccinated and vaccine-naïve participants (Table 1). The mean age of the enrolled participants was 19.7 years (standard deviation ± 2.56) and 51% were males.

3.2. Immunogenicity

3.2.1. Antibody persistence

Four years after 2-dose 4CMenB vaccination, hSBA titers ≥4 in the Primed_4y group were observed in 30%, 84%, 9% and 75% of participants for fHbp, NadA, PorA and NHBA, respectively (Table 2). In the Primed_7.5y participants, 7.5 years after 2-dose 4CMenB vaccination, hSBA titers ≥4 were observed in 44%, 84%, 29% and 81% of participants for fHbp, NadA, PorA and NHBA, respectively (Table 2). Similar observations were made for the percentage of participants with hSBA titers ≥5 (Supplementary table 1).

In both previously vaccinated groups, unadjusted hSBA GMTs decreased over time, but remained above pre-primary vaccination values at 4 and 7.5 years post-primary series, except for NHBA and PorA in the Primed_7.5y group (Table 3). Moreover, adjusted hSBA GMTs for all antigens (except NHBA in the Primed_7.5y group) in previously-vaccinated participants were higher than in vaccine-naïve participants (Table 2).

GMRs (previously vaccinated participants compared to vaccine-naïve participants) were 2.1 (fHbp), 20.0 (NadA), 1.3 (PorA) and 1.3 (NHBA) at 4 years post-vaccination in the Primed_4y group and 3.0 (fHbp), 14.0 (NadA), 1.7 (PorA) and 1.2 (NHBA) at 7.5 years post-vaccination in the Primed_7.5y group (Supplementary Table 2).

3.2.2. Booster response

In the primed groups, at 1 month post-booster dose, the percentages of participants with hSBA titer ≥4 were 93–100% for all antigens; while the percentages of vaccine-naïve participants with hSBA titers ≥4 post-first dose were significantly lower for all antigens except NHBA in the Primed_7.5y group, ranging between 41% and 92% (Supplementary Fig. 2; Supplementary Table 2). hSBA GMTs were also higher in primed participants at 1 month post-booster dose compared to hSBA GMTs post-first dose in vaccine-naïve participants. hSBA GMTs increased post-booster compared to pre-booster 4.7–100-fold in the Primed_4y group and 5.2–64-fold in the Primed_7.5y group, for all vaccine antigens. At 1 month post-first dose, hSBA GMTs increased 2.4–25-fold in the Naïve_4y group and 2.6–16-fold in the Naïve_7.5y group, for all 4CMenB antigens (Supplementary Table 3). When analyzed by country, the percentages of participants with hSBA titers ≥4 and GMTs were similar between participants from Canada and Australia (Supplementary Table 4).

3.2.3. Kinetics of antibody response

The percentage of previously vaccinated participants with hSBA titers ≥4 remained similar to pre-booster against all strains at 3 days post-booster, increased at 7 days post-booster and remained unchanged or further increased at 1 month post-booster dose (Fig. 3).

Overall, the percentage of vaccine-naïve participants with hSBA ≥4 remained similar to pre-first dose values at 3 days post-dose 2, increased at 7 days post-dose 2, and remained unchanged at 1 month post-dose 2 for all antigens (Fig. 3).

In all participants, at 3 days post-booster or post-dose 2, hSBA GMTs remained similar to those before vaccination in the extension study. In general, GMTs increased at 7 days and 1 month post-vaccination (Supplementary Table 5). The proportion of participants with ≥4-fold increase in hSBA titers for previously vaccinated participants (post-booster vs pre-booster dose) ranged between 48% (NHBA) and 98% (fHbp and NadA); and for vaccine-naïve participants (post-second dose vs pre-first dose) between 7% (NHBA) and 72% (NadA) (Supplementary Table 6). Similar observations were made for the percentage of participants with hSBA titers ≥5 (Supplementary table 7).

3.2.4. Immune responses in vaccine-naïve participants

In the FAS booster, pre-first dose, in the Naïve_4y group, hSBA titers ≥4 were observed for 4%, 6%, 0% and 63% participants for fHbp, NadA, PorA and NHBA, respectively; 1 month post-first dose, they increased to 81%, 87%, 41% and 84%. hSBA GMTs increased at least 2.4-fold for all antigens. Post-dose 2 (PPS Kinetics), the percentages of participants with hSBA titer ≥4 increased to 99% and 100% for fHbp and NadA, 82% for PorA, and 91% for NHBA. hSBA GMTs increased 52-fold for fHbp; 204-fold for NadA; 11-fold for PorA and 3.2-fold for NHBA.

Table 1
Study population demographics and baseline characteristics (all enrolled set).

	Group Primed_4y N = 145	Group Primed_7.5y N = 131	Group Primed N = 276	Group Naïve _4y N = 105	Group Naïve _7.5y N = 150	Group Naïve N = 255	Total N = 531
Age, years							
Mean ± SD	18.0 ± 1.88	21.2 ± 1.73	19.5 ± 2.42	17.5 ± 1.85	21.7 ± 1.56	20.0 ± 2.69	19.7 ± 2.56
Median (minimum; maximum)	18.0 (15; 22)	21.0 (18; 24)	20.0 (15; 24)	17.0 (15; 21)	22.0 (17; 24)	21.0 (15; 24)	20.0 (15; 24)
Age at enrollment in parent study, years							
Mean ± SD	13.7 ± 1.85	14.1 ± 1.74	–	–	–	–	–
Median (minimum; maximum)	14.0 (11; 17)	14.0 (11; 17)	–	–	–	–	–
Age group, n (%)							
Adolescents (12–17 years)	65 (45%)	0	65 (24%)	56 (53%)	1 (1%)	57 (22%)	122 (23%)
Adults (≥18 years)	80 (55%)	131 (100%)	211 (76%)	49 (47%)	149 (99%)	198 (78%)	409 (77%)
Sex, n (%)							
Male	80 (55%)	63 (48%)	143 (52%)	51 (49%)	76 (51%)	127 (50%)	270 (51%)
Female	65 (45%)	68 (52%)	133 (48%)	54 (51%)	74 (49%)	128 (50%)	261 (49%)
Race, n (%)							
White	98 (68%)	0	98 (36%)	74 (70%)	0	74 (29%)	172 (32%)
Black or African American	3 (2%)	0	3 (1%)	2 (2%)	0	2 (1%)	5 (1%)
Asian	22 (15%)	0	22 (8%)	18 (17%)	0	18 (7%)	40 (8%)
American Indian or Alaska Native	11 (8%)	0	11 (4%)	1 (1%)	0	1 (<1%)	12 (2%)
Native Hawaiian or other Pacific Islander	3 (2%)	0	3 (1%)	8 (8%)	0	8 (3%)	11 (2%)
Other	8 (6%)	131 (100%)	139 (50%)	2 (2%)	150 (100%)	152 (60%)	291 (55%)
Weight, kg (mean ± SD)	72.4 ± 18.42	69.1 ± 16.30	70.8 ± 17.49	70.6 ± 18.17	67.8 ± 13.18	68.9 ± 15.46	69.9 ± 16.56
Height, cm (mean ± SD)	170.7 ± 9.78	166.5 ± 8.71	168.7 ± 9.51	170 ± 10.06	167.5 ± 9.21	168.5 ± 9.63	168.6 ± 9.56
Years since last vaccination in parent study							
Mean ± SD	4.27 ± 0.08	6.91 ± 0.34	5.53 ± 1.34	–	–	–	–
Median (minimum; maximum)	4.27 (4.08; 4.43)	6.92 (6.21; 7.93)	4.42 (4.08; 7.93)	–	–	–	–
Country of enrollment, n (%)							
Australia	38 (26%)	0	38 (14%)	25 (24%)	0	25 (10%)	63 (12%)
Canada	107 (74%)	0	107 (39%)	80 (76%)	0	80 (31%)	187 (35%)
Chile	0	131 (100%)	131 (47%)	0	150 (100%)	150 (59%)	281 (53%)

Groups Primed_4y and Primed_7.5y, previously vaccinated participants; groups Naïve_4y and Naïve_7.5y, vaccine-naïve participants; N, number of participants in each group; n (%), number (percentage) of participants in a certain category; SD, standard deviation; -, not applicable.

Table 2
Percentage of participants with hSBA titer ≥4 and geometric mean titers, at 1 month, 4 and 7.5 years after the last dose of 4CMenB vaccination in the parent studies and at baseline in vaccine-naïve participants - FAS Persistence.

			Primed_4y		Naïve_4y		Primed_7.5y		Naïve_7.5y	
			N	Value (95%) CI)	N	Value (95% CI)	N	Value (95% CI)	N	Value (95% CI)
fHbp	% ≥4	1 month post-primary vaccination in PS	144	99.0 (95.1–99.83)			131	100 (97.2–100)		
		Pre-booster/first dose	144	30.0 (22.5–38.0)	105	5.0 (1.6–10.8)	131	44.0 (35.6–53.2)	150	13.0 (7.8–19.1)
	GMT	1 month post-primary vaccination in PS	144	99 (82–119)			131	197 (165–235)		
		Pre-booster/first dose	144	2.43 (2.04–2.89)	105	1.14 (0.93–1.40)	131	4.51 (3.57–5.69)	150	1.52 (1.23–1.90)
NadA	% ≥4	1 month post-primary vaccination in PS	134	100 (97.3–100)			120	100 (97.0–100)		
		Pre-booster/first dose	134	84.0 (77.0–90.0)	100	7.0 (2.9–13.9)	120	84.0 (76.4–90.2)	139	24.0 (16.9–31.7)
	GMT	1 month post-primary vaccination in PS	134	180 (153–211)			120	606 (492–746)		
		Pre-booster/first dose	134	24 (19–30)	100	1.20 (0.91–1.58)	120	31 (23–42)	139	2.30 (1.75–3.04)
NHBA	% ≥4	1 month post-primary vaccination in PS	143	70.0 (61.7–77.4)			131	98.0 (94.6–99.81)		
		Pre-booster/first dose	143	75.0 (66.9–81.7)	105	64.0 (53.9–73.0)	131	81.0 (73.1–87.3)	150	79.0 (72.0–85.5)
	GMT	1 month post-primary vaccination in PS	140	10 (7.65–14)			131	66 (53–81)		
		Pre-booster/first dose	140	13 (9.86–18)	105	10 (7.13–15)	131	22 (16–29)	150	18 (14–24)
PorA	% ≥4	1 month post-primary vaccination in PS	144	82.0 (74.7–87.9)			129	99.0 (95.8–99.98)		
		Pre-booster/first dose	144	9.0 (4.9–14.9)	105	0.0 (0.0–3.5)	129	29.0 (21.1–37.3)	148	14.0 (9.0–20.9)
	GMT	1 month post-primary vaccination in PS	144	11 (8.67–14)			129	93 (75–117)		
		Pre-booster/first dose	144	1.31 (1.17–1.45)	105	1.01 (0.89–1.14)	129	2.56 (2.07–3.17)	148	1.50 (1.23–1.84)

hSBA, human serum bactericidal antibody assay; 4CMenB, meningococcal group B vaccine; FAS, full analysis set; N, maximum number of participants with available results; CI, confidence interval; fHbp, factor H-binding protein; NadA, Neisseria adhesin A; NHBA, Neisserial heparin-binding antigen; PorA, porin A; groups Primed_4y and Primed_7.5y previously vaccinated participants from parent studies (2 vaccine doses, 1 month apart); groups Naïve_4y and Naïve_7.5y, vaccine-naïve participants in the extension study; PS, parent study; ES, extension study

In the FAS booster, pre-first dose, in the Naïve_7.5y group, hSBA titers ≥4 were observed for 13%, 22%, 14% and 79% of participants for fHbp, NadA, PorA and NHBA, respectively; 1 month post-first dose they increased to 81%, 84%, 62% and 93%. One-month post-dose 2 (PPS Kinetics), the percentages increased to 100% for fHbp, 98% for NadA, 79% for PorA, and 95% for NHBA. hSBA GMTs increased 41-fold for fHbp; 123–fold for NadA; 11-fold for PorA and 3.2-fold for NHBA, 1 month post-second dose.

Table 3
Unadjusted geometric mean hSBA titers in previously vaccinated participants at baseline and 1 month after the last vaccination in parent studies and at baseline and 1 month after the booster vaccination in the extension study – FAS Booster.

		Group Primed_4y		Group Primed_7.5	
		N	GMT (95% CI)	N	GMT (95% CI)
fHbp	Pre-primary vaccination	141	1.05 (1.00–1.11)	127	2.36 (1.86–2.99)
	1 month post-primary vaccination in PS	141	102 (86–121)	127	202 (170–240)
	Pre-booster dose	141	2.38 (1.94–2.93)	127	4.65 (3.45–6.27)
	1 month post-booster dose in ES	141	162 (132–198)	127	269 (223–325)
NadA	Pre-primary vaccination	124	1.20 (1.07–1.34)	102	1.77 (1.40–2.24)
	1 month post-primary vaccination in PS	124	181 (156–211)	102	579 (462–727)
	Pre-booster dose	118	22 (16–30)	93	31 (21–44)
	1 month post-booster dose in ES	124	2421 (1981–2959)	102	1951 (1629–2337)
NHBA	Pre-primary vaccination	141	3.03 (2.40–3.83)	127	13 (9.85–18)
	1 month post-primary vaccination in PS	138	11 (8.25–14)	127	65 (53–81)
	Pre-booster dose	140	13 (9.99–17)	127	22 (16–29)
	1 month post-booster dose in ES	141	65 (55–76)	127	113 (92–138)
PorA	Pre-primary vaccination	142	1.04 (0.98–1.10)	120	2.06 (1.64–2.58)
	1 month post-primary vaccination in PS	142	11 (8.79–13)	120	93 (74–117)
	Pre-booster dose	142	1.32 (1.15–1.50)	118	2.48 (1.90–3.23)
	1 month post-booster dose in ES	142	29 (24–36)	120	41 (32–52)

hSBA, human complement serum bactericidal antibody assay; FAS, full analysis set; groups Primed_4y and Primed_7.5y, previously vaccinated participants; fHbp, factor H-binding protein; NadA, Neisseria adhesin A; NHBA, Neisserial heparin-binding antigen; PorA, porin A; PS, parent study (2 doses, 1 month apart); ES, extension study; N, maximum number of participants with available results; GMT, geometric mean titer; CI, confidence interval.

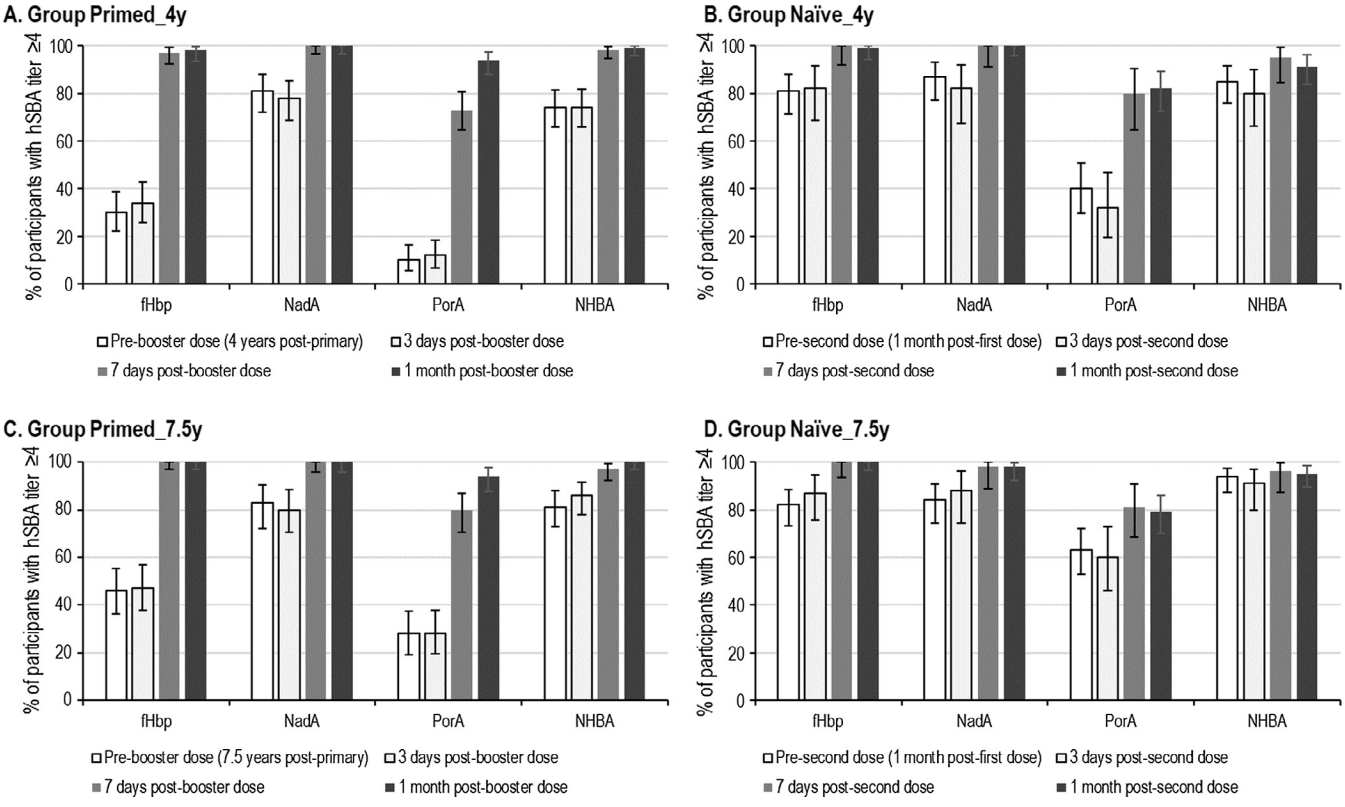

Fig. 3. Percentages of participants with hSBA titer ≥4 after booster/2-dose vaccination with 4CMenB – PPS Kinetics. hSBA, human complement serum bactericidal antibody assay; 4CMenB, meningococcal group B vaccine; PPS, per-protocol set; groups Primed_4y and Primed_7.5y previously vaccinated participants from parent studies; groups Naïve_4y and Naïve_7.5y, vaccine-naïve participants in the extension study; fHbp, factor H-binding protein; NadA, Neisseria adhesin A; PorA, porin A; NHBA, Neisserial heparin-binding antigen. Note: Error bars depict 95% confidence interval.

3.3. Safety and reactogenicity

Overall, almost all participants experienced at least 1 solicited AE post-vaccination. Within 7 days post-any vaccination, ≥97% of participants reported at least 1 solicited local AE and ≥75% reported at least 1 solicited systemic AE. Pain was the most frequently reported solicited local AE (98%), while fatigue (≥55%) and headache (≥49%) were the most frequently reported solicited systemic AEs (Table 4).

Overall, unsolicited AEs were reported by 32% of previously vaccinated participants and 51% of vaccine-naïve participants, 30 days post-any vaccination.

One SAE, appendicitis, was reported by 1 vaccine-naïve participant post-first vaccine dose. This SAE was considered as unrelated

Table 4
Frequency of solicited adverse events (AEs), unsolicited AEs and serious AEs, after any 4CMenB dose.[a]

	Group Primed	Group Naïve
Solicited AEs	**N = 266**	**N = 254**
Local reactions		
Any	258 (97%)	250 (98%)
Induration		
Any	54 (21%)	43 (17%)
Severe	1 (1%)	1 (1%)
Swelling		
Any	60 (23%)	43 (17%)
Severe	1 (1%)	0
Erythema		
Any	54 (21%)	29 (11%)
Severe	7 (3%)	2 (1%)
Pain		
Any	258 (98%)	250 (98%)
Severe	71 (27%)	63 (25%)
Systemic reactions		
Any	203 (76%)	191 (75%)
Nausea		
Any	56 (21%)	51 (20%)
Severe	4 (2%)	5 (2%)
Fatigue		
Any	155 (58%)	140 (55%)
Severe	26 (10%)	23 (9%)
Myalgia		
Any	120 (45%)	98 (39%)
Severe	21 (8%)	12 (5%)
Arthralgia		
Any	84 (32%)	63 (25%)
Severe	13 (5%)	10 (4%)
Headache		
Any	146 (55%)	125 (49%)
Severe	18 (7%)	19 (7%)
Fever		
Any (≥38 °C)	16 (6%)	9 (4%)
High fever (≥39.5 °C)	0	0
Unsolicited AEs	**N = 275**	**N = 255**
Any[b]	87 (32%)	131 (51%)
General disorders and administration site conditions	40 (15%)	61 (24%)
Infection and infestations	30 (11%)	62 (24%)
Gastrointestinal disorders	2 (1%)	13 (5%)
Musculoskeletal and connective tissue disorders	9 (3%)	7 (3%)
Nervous system disorders	9 (3%)	24 (9%)
Possibly or probably related unsolicited AEs	45 (16%)	80 (31%)
Medically-attended AEs	17 (6%)	34 (13%)
Serious AEs	0	1 (1%)

Primed, previously vaccinated participants; group Naïve, vaccine-naïve participants; N, number of participants in a certain group.
[a] Single dose in the Primed group and any of the 2 vaccine doses in the Naïve group.
[b] The grouping of unsolicited AEs was done according to system organ class using the medical dictionary for regulatory activities (MedDRA) dictionary.

to the study vaccine by the investigator and was resolved after 25 days. No death was reported in the study.

4. Discussion

For the first time, we describe long-term antibody levels following 2 primary doses of 4CMenB in adolescents, measuring the percentages of individuals with protective antibody titers up to 7.5 years after primary vaccination. Also, we describe the response to a booster dose in late adolescence and early adulthood, providing key information for decisions on booster vaccination.

The results of this study show that up to 7.5 years post-primary series, antibody levels generally declined for all vaccine antigens. hSBA titers ≥4 were retained by 9% (for PorA) to 84% (for NadA) of Australian and Canadian participants, up to 4 years, and by 29% (for PorA) to 84% (for NadA) of Chilean participants, up to 7.5 years post-primary vaccination with 4CMenB. These levels were lower than those observed 18–23 months after vaccination with 2 doses of 4CMenB in Chile (75–95%) [18]. Importantly, the percentage of participants with hSBA titers ≥4 in previously vaccinated participants was higher than that of vaccine-naïve participants against all vaccine antigens, except for NHBA, for which similarly high levels were observed in the 2 groups.

The observed higher persistence of antibody levels against 4CMenB antigens in the Chilean cohort compared with that in the Australian and Canadian population, despite a longer follow-up period, is in line with the apparent mounting of a stronger immune response noted at 1 month post-primary vaccination. Moreover, higher pre-primary vaccination titers consequently resulted in higher immune response following vaccination. Several potential hypotheses for the differences between Chilean and Australian/Canadian adolescents can be formulated, which would require novel study designs to be addressed. Of note, differences between pre-vaccination hSBA titers in UK university students and Chilean adolescents were previously observed, with baseline titers being higher in the case of the European trial [20]. It must also be noted that in our studies, immunogenicity was assessed at different laboratories during the parent and extension studies. Despite the consensus on the requirement of laboratory reporting to unify laboratory testing, laboratory assays remain subject to variations and interlaboratory comparisons should therefore be interpreted with caution. Other factors, such as environmental and behavioral ones (for instance, exposure to daily smoking or household crowding) can also point to different exposure to the pathogen and potentially account for the difference observed in pre-vaccination titers between Chilean and Australian/Canadian participants. In a study assessing immune responses to a *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine administered to Chilean participants, environmental factors were also associated with enhanced responses to vaccination, when compared with infants in the US or elsewhere [25]. However, a similar impact is unlikely in the case of 4CMenB vaccination, since to date, no association between environmental factors and immune responses has been observed in the course of any clinical trial.

4CMenB is a combination of 4 antigens, selected to provide broad protection against the majority of MenB-related disease with a high proportion of MenB strains expressing 2 or more vaccine antigens [26]. Immunogenicity was measured for each antigen separately, using representative *N. meningitidis* strains recognized only by antibodies induced by a single vaccine component. Such assessments may underestimate overall immunogenicity since they do not account for the cooperative activity between different vaccine-induced antibodies. For the many strains expressing more than one vaccine antigen, vaccine coverage may be maintained even if the expression of one antigen is below the threshold deemed predictive of killing [10]. Further studies are needed to accurately assess how laboratory data on antibody response to reference strains can be extrapolated to real-life impact on IMD. The disadvantages and deriving limitations of the laboratory assays currently used for the evaluation of immune response to meningococcal vaccinations are well known [27]. Also, immunogenicity data in our study is generated from different populations, for which baseline characteristics, and therefore the elicited immune response to 4CMenB vaccination, can vary. The correlation between the currently accepted cut-off for protective antibody levels and effective protection against infection and IMD is also not clearly defined. Nevertheless, the existence of such a correlation is supported by emerging real-life data on the impact of 4CMenB on MenB-caused IMD incidence. In the UK, a coverage of 88% across MenB strains is predicted by hSBA using pooled post-

4-dose immunization infant sera [28], while a 2-dose field effectiveness of 83% against all MenB cases was derived from real-life data following the introduction of 4CMenB in the national immunization plan [22]. Assessment of alternative measures, such as coverage against circulating strains, is also informative for estimations of vaccine impact and effectiveness. Long-term surveillance data and direct comparison to laboratory estimates of protection will add important information to the understanding of the true effect of vaccination on IMD.

At 4 and 7.5 years post-primary vaccination, antibody levels declined, but were higher than in vaccine-naïve participants, for all antigens except NHBA which was relatively high in both groups. The highest persistence was observed for NadA, followed by fHbp and PorA. For NHBA, the percentage of participants with hSBA titers ≥4 was comparable at 1 month post-primary vaccination and at 4 and 7.5 years and similar antibody levels were observed for previously primed and vaccine-naïve participants before vaccination in the current study. As NHBA, a surface-exposed lipoprotein, is highly conserved in all strains of pathogenic MenB and commensal Neisserial species tested so far, the higher initial titers may be due to exposure to circulating strains [10,29].

Robust immunological responses were observed following booster vaccination at 4 years (94–100%) and 7.5 years (93–100%) post-primary vaccination. A high proportion of vaccine-naïve participants also achieved hSBA titers ≥4 post-administration of 2 vaccine doses (79–100%).

The administration of a 4CMenB booster dose in the previously vaccinated participants showed a more robust immune response with higher increase of hSBA titers, as compared to the response to a first dose in vaccine-naïve participants of similar age. This suggests that despite a decline of bactericidal antibodies over time, previous vaccination with 4CMenB resulted in priming for immune memory and induced an anamnestic response in most individuals. The booster response was similar irrespective of the persistence period (4 or 7.5 years) post-primary vaccination in the parent studies. Two doses of 4CMenB administered 1 month apart to 15–24-year-old vaccine-naïve participants induced an overall robust antibody response for all vaccine antigens (79–100%) with evidence of an early response from 1 month post-first dose (41–93%). Quantitative comparisons with other age-groups are not possible, due to the differences in vaccination schedules and more importantly, in immunity between infants/children and adolescents/adults. However, despite declines in antibody levels following priming with 4CMenB, infants and young children also show robust immune responses following an additional booster dose of 4CMenB [23].

The study also investigated the kinetics of the antibody response post-booster in previously vaccinated participants, and post-second dose in vaccine-naïve participants. In both groups, no significant increase in antibody levels was noted at day 3, but a robust response was observed at 7 days post-vaccination with 2 doses or a booster dose of 4CMenB, for all vaccine antigens. GMTs remained at high levels or further increased at 1 month post-booster (previously vaccinated participants) or post-second dose (vaccine-naïve participants). For all antigens, hSBA titers were higher at 1 month post-booster than at 1 month post-second dose in vaccine-naïve participants. However, as observed in a previous study [16], first dose response suggests that 4CMenB could provide some protection within 4 weeks from vaccination, even in previously unvaccinated individuals, in case of epidemic outbreaks.

The vaccine was generally well-tolerated although solicited AEs were reported by most participants. The incidence of unsolicited AEs was similar between the previously vaccinated and vaccine-naïve participants. In line with previous studies, most solicited local AEs and unsolicited AEs were mild to moderate in intensity, and resolved within a few days. Studies on 4CMenB that have enrolled participants of 10 years and older showed that the vaccine, albeit reactogenic, is well-tolerated with local and systemic reactions reported in similar proportions to those observed in this study. Pain was the most common local reaction, reported in ≥90% of the vaccinees, also comparable to similar studies [16,17]. Myalgia was the most common systemic reaction reported in other trials in adolescents and young adults [17,19], whereas in our study, headache and fatigue were reported as the most common systemic reactions. As expected for an adolescent population in light of previous studies [16,17], fever was not a common systemic reaction, and no participant reported high fever. No new safety clinical concerns were reported during this study.

5. Conclusion

Antibody levels against any one of the 4 4CMenB antigens remained above the protective threshold for 9–84% of adolescents, at 4–7.5 years after a 2-dose primary-vaccination series. Although antibody levels declined over time, a higher proportion of primed participants had hSBA titers ≥4, at 4 and 7.5 years post-primary vaccination, compared with vaccine-naïve participants, except for NHBA, which was relatively high for vaccinated and unvaccinated individuals. A more robust immune response was generally observed after a booster dose compared to a first dose in vaccine-naïve population, demonstrating priming for immune memory and induction of an anamnestic response in a young adult population. Our study provides important information that will help to inform the choice of vaccination strategies to protect against IMD in adolescents and young adults.

Trademark statement

Bexsero is a trademark of the GSK group of companies.

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Declaration of interest

DT is an GSK employee and holds shares in the GSK group of companies as part of her employee remuneration. DD'A was an employee of the GSK group of companies. PRh owns stock options of the GSK group of companies. TN, HM, FdL, KP and PRh received institutional grants for conducting studies from GlaxoSmithKline Biologicals SA. TN and HM also received institutional grants to conduct clinical trials from Pfizer and Novartis. HM also acknowledges

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Author contribution

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors reviewed and commented critically on drafts of the manuscript for important intellectual content and gave final approval to submit for publication. DT, DD'A, TN, HM, PRi, MES, MO'R and KP contributed to the study conception and design. MO'R, TN, HM, PRi, MF, AG, PRh, HG, KH, FdL, MES, SH and KP contributed to the acquisition of data. DD'A, TN, HM, PRi, AG, FdL, DT, MES, MÓR and KP contributed to the analysis and interpretation of data and manuscript writing. DD'A provided statistical expertise.

Data sharing statement

The results summary for this study (GSK study number 205218 – NCT02446743) is available on the GSK Clinical Study Register and can be accessed at www.gsk-clinicalstudyregister.com. For interventional studies that evaluate our medicines, anonymized patient-level data will be made available to independent researchers, subject to review by an independent panel, at www.clinicalstudydatarequest.com within six months of publication. To protect the privacy of patients and individuals involved in our studies, GSK does not publicly disclose patient-level data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.12.059.

References

[1] Halperin SA, Bettinger JA, Greenwood B, Harrison LH, Jelfs J, Ladhani SN, et al. The changing and dynamic epidemiology of meningococcal disease. Vaccine 2012;30(Suppl 2):B26–36. https://doi.org/10.1016/j.vaccine.2011.12.032.

[2] Pelton SI. The global evolution of meningococcal epidemiology following the introduction of meningococcal vaccines. J Adolesc Health 2016;59:S3–S11. https://doi.org/10.1016/j.jadohealth.2016.04.012.

[3] Banzhoff A. Multicomponent meningococcal B vaccination (4CMenB) of adolescents and college students in the United States. Ther Adv Vaccines 2017;5:3–14. https://doi.org/10.1177/2051013616681365.

[4] Archer BN, Chiu CK, Jayasinghe SH, Richmond PC, McVernon J, Lahra MM, et al. Epidemiology of invasive meningococcal B disease in Australia, 1999–2015: priority populations for vaccination. Med J Aust 2017;207:382–7.

[5] De Wals P, Deceuninck G, Lefebvre B, Tsang R, Law D, De Serres G, et al. Impact of an immunization campaign to control an increased incidence of Serogroup B meningococcal disease in one region of Quebec, Canada. Clin Infect Dis 2017;64:1263–7. https://doi.org/10.1093/cid/cix154.

[6] Ibarz-Pavon AB, Lemos AP, Gorla MC, Regueira M, Gabastou JM. Laboratory-based surveillance of *Neisseria meningitidis* isolates from disease cases in Latin American and Caribbean countries, SIREVA II 2006–2010. PLoS One 2012;7:e44102. https://doi.org/10.1371/journal.pone.0044102.

[7] Australian Government. Department of Health. Invasive meningococcal disease national surveillance report with a focus on MenW. 31 December 2017; 2017.

[8] Pan-Canadian Public Health Network Partners in Public Health. 2014. The Recommended Use of the Multicomponent Meningococcal B (4CMenB) Vaccine in Canada. Available from: <https://www.canada.ca/en/public-health/services/immunization/national-advisory-committee-on-immunization-naci/recommended-use-multicomponent-meningococcal-b-4cmenb-vaccine-canada.html> [accessed 2017 Aug 30].

[9] de Salud Ministerio, de Chile Gobierno. Departamento de Epidemiología. Enfermedad meningocóccica: Informe epidemiológico; 2015.

[10] Toneatto D, Pizza M, Masignani V, Rappuoli R. Emerging experience with meningococcal serogroup B protein vaccines. Expert Rev Vaccines 2017;16:433–51. https://doi.org/10.1080/14760584.2017.1308828.

[11] Donnelly J, Medini D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, et al. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proc Natl Acad Sci USA 2010;107:19490–5. https://doi.org/10.1073/pnas.1013758107.

[12] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307–26.

[13] Safadi MA, Bettinger JA, Maturana GM, Enwere G, Borrow R. Evolving meningococcal immunization strategies. Expert Rev Vaccines 2015;14:505–17. https://doi.org/10.1586/14760584.2015.979799.

[14] Vetter V, Baxter R, Denizer G, Safadi MA, Silfverdal SA, Vyse A, et al. Routinely vaccinating adolescents against meningococcus: targeting transmission & disease. Expert Rev Vaccines 2016;15:641–58. https://doi.org/10.1586/14760584.2016.1130628.

[15] Ali A, Jafri RZ, Messonnier N, Tevi-Benissan C, Durrheim D, Eskola J, et al. Global practices of meningococcal vaccine use and impact on invasive disease. Pathog Glob Health 2014;108:11–20. https://doi.org/10.1179/2047773214y.0000000126.

[16] Santolaya ME, O'Ryan ML, Valenzuela MT, Prado V, Vergara R, Munoz A, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. Lancet 2012;379:617–24. https://doi.org/10.1016/S0140-6736(11)61713-3.

[17] Perrett KP, McVernon J, Richmond PC, Marshall H, Nissen M, August A, et al. Immune responses to a recombinant, four-component, meningococcal serogroup B vaccine (4CMenB) in adolescents: a phase III, randomized, multicentre, lot-to-lot consistency study. Vaccine 2015;33:5217–24. https://doi.org/10.1016/j.vaccine.2015.06.103.

[18] Santolaya ME, O'Ryan M, Valenzuela MT, Prado V, Vergara RF, Munoz A, et al. Persistence of antibodies in adolescents 18–24 months after immunization with one, two, or three doses of 4CMenB meningococcal serogroup B vaccine. Hum Vaccin Immunother 2013;9:2304–10. https://doi.org/10.4161/hv.25505.

[19] Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, et al. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: an observer-blind, phase 3 randomised clinical trial. Lancet 2014;384:2123–31. https://doi.org/10.1016/s0140-6736(14)60842-4.

[20] Read RC, Dull P, Bai X, Nolan K, Findlow J, Bazaz R, et al. A phase III observer-blind randomized, controlled study to evaluate the immune response and the correlation with nasopharyngeal carriage after immunization of university students with a quadrivalent meningococcal ACWY glycoconjugate or serogroup B meningococcal vaccine. Vaccine 2017;35:427–34. https://doi.org/10.1016/j.vaccine.2016.11.071.

[21] Nolan T, O'Ryan M, Wassil J, Abitbol V, Dull P. Vaccination with a multicomponent meningococcal B vaccine in prevention of disease in adolescents and young adults. Vaccine 2015;33:4437–45. https://doi.org/10.1016/j.vaccine.2015.06.011.

[22] Parikh SR, Andrews NJ, Beebeejaun K, Campbell H, Ribeiro S, Ward C, et al. Effectiveness and impact of a reduced infant schedule of 4CMenB vaccine against group B meningococcal disease in England: a national observational cohort study. Lancet 2016;388:2775–82. https://doi.org/10.1016/s0140-6736(16)31921-3.

[23] Martinon-Torres F, Carmona Martinez A, Simko R, Infante Marquez P, Arimany JL, Gimenez-Sanchez F, et al. Antibody persistence and booster responses 24–36 months after different 4CMenB vaccination schedules in infants and children: a randomised trial. J Infect 2018;76:258–69. https://doi.org/10.1016/j.jinf.2017.12.005.

[24] Mak PA, Santos GF, Masterman KA, Janes J, Wacknov B, Vienken K, et al. Development of an automated, high-throughput bactericidal assay that measures cellular respiration as a survival readout for Neisseria meningitidis. Clin Vaccine Immunol 2011;18:1252–60. https://doi.org/10.1128/cvi.05028-11.

[25] Levine OS, Granoff DM, Lagos R, Fritzell B, Levine MM. Factors associated with superior antibody responses to a single dose of Haemophilus influenzae type b-tetanus toxoid conjugate vaccine administered to Chilean infants at 2 months of age. Vaccine 1997;15:325–8.

[26] Wang X, Cohn A, Comanducci M, Andrew L, Zhao X, MacNeil JR, et al. Prevalence and genetic diversity of candidate vaccine antigens among invasive Neisseria meningitidis isolates in the United States. Vaccine 2011;29:4739–44. https://doi.org/10.1016/j.vaccine.2011.04.092.

[27] McIntosh ED, Broker M, Wassil J, Welsch JA, Borrow R. Serum bactericidal antibody assays - the role of complement in infection and immunity. Vaccine 2015;33:4414–21. https://doi.org/10.1016/j.vaccine.2015.07.019.

[28] Frosi G, Biolchi A, Lo Sapio M, Rigat F, Gilchrist S, Lucidarme J, et al. Bactericidal antibody against a representative epidemiological meningococcal serogroup B panel confirms that MATS underestimates 4CMenB vaccine strain coverage. Vaccine 2013;31:4968–74. https://doi.org/10.1016/j.vaccine.2013.08.006.

[29] Martinon-Torres F, Safadi MAP, Martinez AC, Marquez PI, Torres JCT, Weckx LY, et al. Reduced schedules of 4CMenB vaccine in infants and catch-up series in children: Immunogenicity and safety results from a randomised open-label phase 3b trial. Vaccine 2017;35:3548–57. https://doi.org/10.1016/j.vaccine.2017.05.023.